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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/16, 39/10, C07K 14/235

A1

(11) International Publication Number: WO 96/17620

(43) International Publicati n Date: 13 June 1996 (13.06.96)

(21) International Application Number: PCT/US95/16450

(22) International Filing Date: 4 December 1995 (04.12.95)

(30) Priority Data:
111866 4 December 1994 (04.12.94) IL

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(81) Designated States: CA, JP, MX, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF AUTOIMMUNE DISEASES COMPRISING THE B-OLIGOMER OF PERTUSSIS TOXIN OR ITS SUBUNITS

(57) Abstract

The invention provides the use of a protein selected from the B-oligomer of pertussis toxin, an individual subunit S2, S3, S4 or S5 thereof, or a combination of said subunits, for the preparation of pharmaceutical compositions comprising them for the treatment of autoimmune diseases.

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PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF AUTOIMMUNE DISEASES COMPRISING THE B-OLIGOMER OF PERTUSSIS TOXIN OR ITS SUBUNITS

Field of the Invention

The present invention is generally in the field of agents that may be used for the treatment of autoimmune diseases, and more particularly relates to pharmaceutical compositions comprising the B-oligomer of pertussis toxin or one of its subunits S2, S3, S4 or S5, or combinations thereof, useful for protection against autoimmune diseases.

Background of the Invention

The gram-negative bacterium *Bordetella pertussis (B. pertussis)*, the causative agent of whooping cough, produces several virulence factors. Pertussis toxin (PT), the major virulence component of *B. pertussis*, appears to contain an important epitope that leads to the formation of antibodies capable of protecting against the disease. Therefore, PT has been extensively investigated with regard to its possible use in preparing vaccines for whooping cough (Black et al., 1988).

Pertussis toxin is a 105-kDa hexameric protein composed of five distinct non-covalently linked polypeptides designated (in the order of decreasing molecular weight) S1-S5. PT can be divided into two distinct functional units, the enzymatically active toxic A-protomer, consisting of a single polypeptide (S1), and the pentameric B-oligomer (S2, S3, two copies of S4, and S5, i.e. molar ratio1:1:2:1). The B-oligomer is responsible for binding of the toxin to the surface of eukaryotic target cells. The two S4 polypeptides form two distinct heterodimers with S2 and S3, which are in turn held together by S5 (see review by Gierschik, 1992).

The pertussis toxin gene has been cloned and sequenced (Nicosia et al., 1986; European Patent Application EP 0232 229; Locht and Keith, 1986; US Patent 4, 883,761). The individual subunits have been subcloned and expressed in *E. coli* in non-fusion form (Burnette et al.,1988) or as fusion proteins (Nicosia et al., 1987), and tested as antigens for protection against whooping cough.

The development of experimental autoimmune encephalomyelitis (EAE), as well as other autoimmune diseases in experimental animals, can be facilitated by injecting *Bordetella pertussis* concomitantly with inoculation of the autoantigen (Bernard et al., 1992). EAE is a neurological autoimmune disease which can be induced in experimental animals by a single injection of central nervous system (CNS) tissue homogenate or purified myelin antigens such as myelin basic protein (MBP) or proteolipid protein (PLP) in complete Freund's adjuvant (CFA) (Tabira and Kira, 1992). The clinical and pathological features of EAE are reminiscent of multiple sclerosis , and EAE is a well-accepted model for multiple sclerosis. In mice, consistent elicitation of EAE was shown to be facilitated by administration of *B. pertussis* at the time of the encephalitogenic challenge (Munoz, 1985). Pertussis toxin (PT) was shown later to be the component of *B. pertussis* responsible for facilitating disease development, and it is now routinely used in place of *B. pertussis* for enhancement of autoimmune disease in experimental animals (Munoz, 1995).

In an analysis of the possible immunomodulating activity of various bacteria, it was found by the present inventor that *B. pertussis* not only enhances the development of EAE in mice, but can also protect against the disease depending on the time and route of injection (Lehman and Ben-Bun, 1993). The protective activity of *B. pertussis* was subsequently assigned to PT (Ben-Nun et al., 1993).

Pertussis toxin (PT), the major virulence determinant of B. pertussis, is composed of two distinct functional units: the A-protomer consisting of a single polypeptide (S1) that mediates adenosine diphosphate (ADP)-ribosylation of host G proteins, and the B-oligomer, a complex pentamer composed of subunits S2, S3, S4 and S5 in a respective molar ratio of 1:1:2:1, which mediates the binding of the toxin to host tissue by interaction with glycoproteins and glycolipids on many types of eukaryotic cells (Gierschik, 1992). PT was found to have mitogenic and immunoadjuvant properties (Munoz, 1985). The mechanism by which PT can enhance the development of EAE in mice is not yet clear. However, it has been suggested that PT facilitates the access of autoantigen-specific T cells to the CNS by affecting the vascular permeability of the blood-brain barrier (Linthicum et al., 1982).

The mechanism by which PT protects against the development of EAE is also unclear. To further understand how both enhancing and protective activities are mediated by the same, albeit complex. molecule, it is essential to delineate the regions of the PT holomer which may be associated with one or both of these activities.

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Summary of the Invention

It has now been found in accordance with the present invention that the B-oligomer of pertussis toxin or a subunit S2, S3, S4 or S5 thereof, are able to block the development of EAE in mice. Since EAE is a well-established and widely accepted 10 animal model for the study of autoimmune diseases, these findings indicate that the B-oligomer of pertussis toxin or a subunit S2, S3, S4 or S5 thereof, will be useful for the protection against autoimmune diseases in humans.

The present invention thus relates to a pharmaceutical composition comprising as active ingredient the B-oligomer of pertussis toxin or an individual subunit S2, S3, S4 15 or S5 thereof, or a combination of said subunits, and a pharmaceutically acceptable carrier, useful for protection against autoimmune diseases in humans.

Any autoimmune disease can be treated with a pharmaceutical composition of the invention, such as rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus and graft-versus-host disease, and more particularly, 20 multiple sclerosis .

The invention also relates to the use of the B-oligomer of pertussis toxin or an individual subunit S2, S3, S4 or S5 thereof, or a combination of said subunits, for the manufacture of a pharmaceutical composition useful for the protection against autoimmune diseases in humans.

25 Also encompassed by the present invention is a method for the treatment of a patient afflicted with an autoimmune disease which comprises administering to such a patient an effective amount of the B-oligomer of pertussis toxin or an individual subunit S2. S3, S4 or S5 thereof, or a combination of said subunits.

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Description of the Figures

Fig. 1 depicts the nucleotide and amino acid sequence of the subunit S2 of the B-oligomer of pertussis toxin.

Fig. 2 depicts the nucleotide and amino acid sequence of the subunit S3 of the B-oligomer of pertussis toxin.

Fig. 3 depicts the nucleotide and amino acid sequence of the subunit S4 of the B-oligomer of pertussis toxin.

Fig. 4 depicts the nucleotide and amino acid sequence of the subunit S5 of the B-oligomer of pertussis toxin.

Fig. 5 shows protection against the development of EAE by PT administered in oil or in aqueous solution. PT (400ng) in 0.1 ml emulsion of IFA or in aqueous solution (PBS) was injected s.c. in the flanks, 18 days prior to the encephalitogenic challenge. X denotes mortality of all mice in the group.P and M represent incidence of paralysis and mortality, respectively, in each group. a) $p = 0.0002$, and b) $p = 0.0012$, when compared to P or M, respectively, in the combined controls (None + IFA).

Fig. 6 shows that the B-oligomer blocks the development of EAE. B-oligomer (100 ng) or PT (400 ng) emulsified in IFA were injected s.c. 16 days prior to the encephalitogenic challenge. a) $p = 0.001$, and b) $p = 0.001$ when compared to P or M, respectively, in the combined controls (None + IFA).

Fig. 7 shows silver-stained SDS-gel of gel-purified PT subunits. The subunits were isolated by preparative gel electrophoresis and analyzed. Five µg whole PT, 0.4µg purified S1, 0.3µg purified S2, 0.7µg purified S3 and 1.25µg purified S4/S5 were run on the relevant lanes.

Fig. 8 shows differential protective effect of the B-oligomer subunits. 100 ng of B-oligomer or of gel-purified subunits were emulsified in IFA and injected s.c. 20 days before the encephalitogenic challenge. a) $p = 0.0002$, b) $p = 0.0043$, c) $p = 0.0016$, and d) $p = 0.014$ when compared to P in the combined controls (None + IFA); e) $p = 0.001$, f) $p = 0.008$ and g) $p = 0.04$ when compared to M in the combined controls (None + IFA).

Fig. 9 shows that the B-oligomer does not promote the development of EAE. PT (400 ng) or B-oligomer (400 ng) were injected I.V. immediately, and 48 hours after

mice were injected with MSCH/CFA to induce the development of EAE. a) $p = 0.004$, and $p = 0.02$ when compared to P or M, respectively, in the +PT group.

Detailed Description of the Invention

PT administered in oil or in aqueous solution protects mice against the development of EAE (Fig. 5), which is routinely used as a model for organ-specific T-cell mediated autoimmune diseases, suggesting its potential efficacy for therapy of such diseases. However, the use of PT as a therapeutic agent is rather risky in view of its toxicity.

In order to identify regions of PT associated with enhancement or with blocking of disease, both for potential therapeutic use and for a better understanding of the mechanisms of action of PT on autoimmune diseases, the effect of the constitutive units of PT on the development of EAE was investigated according to the present invention. The findings shown herein in the examples that the B-oligomer of PT, which does not contain the toxic S1 monomer, has a remarkable protective effect against EAE (Fig. 6), indicate that the B-oligomer is responsible for the protective activity of PT. Furthermore, the failure of the B-oligomer to enhance the development of EAE (Fig. 9) indicates that the enhancement of autoimmune diseases by PT is likely to be associated with the S1 monomer of PT.

Several features of the B-oligomer of PT make it a potentially useful agent for the therapy of autoimmune diseases: i) it is highly potent in blocking the development of EAE; ii) it is devoid of the enhancing activity of PT; and iii) the B-oligomer, which does not comprise the toxic S1 monomer, is non-toxic in vivo.

The subunits S2, S3, S4 and S5 of the B-oligomer were purified (Fig. 7) and tested for their ability to protect against EAE, in the attempt to assign the protective activity of the B-oligomer to a particular subunit. The findings that each subunit has protective activity (Fig. 8) further indicate that each can be used as a therapeutic agent for autoimmune diseases. Each subunit protected against EAE to a different extent, and the protection conferred was not as complete as that given by the B-oligomer, indicating that various combinations of the subunits in the form of mixtures, e.g. S2+S3, S2+S4,

S2+S3+S5, or as heterodimers, e.g. S2/S4, S3/S4, may be more effective in abolishing disease development.

Hence, the B-oligomer of PT and the subunits of the B-oligomer, individually or in various combinations, are potentially useful agents for the therapy of multiple sclerosis and other autoimmune diseases, such as rheumatoid arthritis, insulin-dependent diabetes mellitus, systemic lupus erythematosus, and graft-versus-host disease.

In preferred embodiments, the pharmaceutical compositions of the invention comprise as active ingredient the B-oligomer, the subunit S2 or S3, a mixture of S2 and S3, or of S2, S3 and S5, or a heterodimer S2/S4 or S3/S4, or a mixture of said heterodimers.

The B-oligomer of PT is commercially available or can be prepared by any method described in the art, such as for example by dissociation of pertussis toxin into the A protomer and the B-oligomer by affinity chromatography (Tamura et al., 1982; and 1983; Burns et al., 1987), or by combining heterodimer S2/S4, heterodimer S3/S4 and subunit S5 at the 1:1:1 molar ratio in 2M urea (Tamura et al., 1983). All attempts to prepare recombinant B-oligomer have not been successful.

The individual subunits S2, S3, S4 and S5, both native and recombinant, are encompassed by the invention. The native subunits can be prepared, for example, by preparative gel electrophoresis as described according to the present invention (Fig. 7) or by gel filtration (Tamura et al., 1982), and the recombinant subunits as described by Nicosia et al, 1987, and Burnette et al., 1988. The heterodimers S2/S4 and S3/S4 are also prepared according to Tamura et al., 1982.

The pharmaceutical compositions of the invention comprise a pharmaceutically acceptable carrier and as active ingredient the B-oligomer of PT or an individual subunit S2, S3, S4 or S5 thereof, or a combination of said subunits. By "combination" of said subunits it is herein included both mixtures of the subunits, for example a mixture of S2 and S3, or S2 and S4, or a heterodimer S2/S4 and S3/S4 associated similarly to the native molecule, or a mixture of said heterodimers.

The pharmaceutical compositions are prepared by mixing the active ingredient with a pharmaceutically acceptable carrier, stabilizers and excipients, and prepared in dosage form, e.g. by lyophilization in dosage vials. They may be administered in all

suitable ways, e.g. intravenously, intramuscularly, subcutaneously, local injection, topical application or per os, as the case may require. The amount of active compound to be administered will depend on the route of administration, the disease to be treated and the condition of the patient.

The invention will now be described in more detail in the following non-limiting examples and their accompanying figures.

EXAMPLES

Materials and Methods

(a) Materials

Pertussis toxin (PT) was obtained from Sigma (St Louis, Mo, USA) and also as a kind gift from Dr. D. Teitelbaum (The Weizmann Institute of Science, Rehovot, Israel). The B-oligomer of PT was obtained from List Biological Laboratories Inc. (Campbell, CA).

(b) Mice

Female SJL/J mice were purchased from Jackson Laboratories (Bar Harbor, Me, USA). All mice were 2-3 months old when used in the experiments.

(c) Induction of EAE

EAE was induced in mice as previously described (Ben-Nun and Lando, 1983). Briefly, 0.1 ml of emulsion prepared from mouse spinal cord homogenate (MSCH, 60 mg/ml) emulsified with an equal volume of CFA enriched for M. tuberculosis H37Ra (5mg/ml) was injected s.c. into the mouse footpads. Immediately after and 48 hrs later, PT was injected intravenously (400 ng/mouse). Where stated in the text, the B-oligomer (400 ng/mouse) was also used in place of PT to evaluate its effect on disease enhancement.

(d) Clinical evaluation, scoring and statistical analysis

Following the encephalitogenic challenge, mice were observed daily for clinical signs of EAE. The severity of the clinical manifestations was scored daily for each individual mouse in the treatment group on a scale of 0-6; 0: no clinical signs, 1: loss of tail tonicity, 2: flaccid tail, 3: hind leg paralysis, 4: hind leg paralysis with hind body

paresis, 5: hind and fore leg paralysis, 6: death. Results presented denote the mean score of the treatment group on each given day after encephalitogenic challenge. At the peak of the clinical disease, on days 12-17 after encephalitogenic challenge, the standard errors (not shown) were less than 16% of the mean clinical score. Each graph also displays the incidence of paralysis (P) and mortality (M) for each treatment group in order to allow a complete evaluation of the clinical course of EAE.

To evaluate the statistical significance of the differences in incidence of paralysis or mortality between treatment and control groups in each experiment, the contingency table analysis was used to calculate the χ^2 values with the Stat View 512+ program. Due to the relatively small number of mice in each group, the *p* values given are of χ^2 with continuity correction.

(e) Protection against EAE

For the induction of protection against the disease, mice were injected s.c. in the flanks with 400 ng of PT, or with 100 ng of B-oligomer or gel-eluted subunits of the B-oligomer, emulsified in incomplete Freund's adjuvant (IFA). A total volume of 0.1 ml was injected 2-3 weeks prior to encephalitogenic challenge. Where indicated, PT was injected in aqueous solution (PBS) s.c. in the flanks, in a total volume of 0.1 ml.

Example 1. Effect of pertussis toxin on the development of EAE

Fig. 5 shows that a single injection of PT prior to the encephalitogenic challenge has a dramatic effect on the subsequent development of EAE induced in SJL/J mice by CNS tissue homogenate. PT was remarkably effective in blocking the development of EAE regardless of whether it was administered s.c. as an oil emulsion or an aqueous solution. These results show that PT can not only facilitate the development of autoimmune diseases as previously shown (Munoz, 1985), but also has a strong protective activity which can completely abolish disease development (Ben-Nun et al., 1993).

Example 2. Effect of the B-oligomer of pertussis toxin on the development of EAE

To identify regions of the PT molecule that can be associated with the enhancing effect or with the protective activity of PT, the A-protomer and the B-oligomer were

investigated. Fig. 6 shows that the B-oligomer, which is devoid of the toxic S1 subunit, is sufficient to protect against the development of EAE. The protection against EAE was total with as little as 50-100 ng of B-oligomer per mouse administered as a single injection. B-oligomer was at least as potent as whole PT in protecting mice against the disease.

Example 3. Purification of PT subunits by elution from preparative SDS-gels and their effect on the development of EAE

In the attempt to assign the protective activity of PT to a particular subunit of the B-oligomer, PT (Sigma) was electrophoresed on preparative SDS-gel according to Laemmli, 1970. Each subunit was located according to stained reference lane on each gel (Fig. 7). The unstained relevant bands were cut out from the gel. Each subunit was eluted from the crushed gel band by passive diffusion, dialysed extensively against double distilled H_2O in the presence or absence of bovine serum albumin, lyophilised and resuspended in PBS.

The B-oligomer, subunits S2, S3 and S4/S5 were tested in vivo for protective activity against the development of EAE. As shown in Fig. 8, each of the subunits had a protective effect. However, although the protective activity of the different subunits was marked, the protection was not as total as that obtained with B-oligomer or whole PT.

Example 4. The B-oligomer does not promote development of EAE in mice

As mentioned above, PT has a conflicting immunomodulatory effect on autoimmune diseases (Ben-Nun et al., 1993). It can facilitate the development of the disease and also protect against it (as shown here). As the B-oligomer was found to be highly effective in protecting against EAE, its possible enhancing effect on the disease was investigated. Fig. 9 shows that in comparison with PT, that facilitated the development of a disease with severe neurological impairment and high mortality, the B-oligomer had no enhancing effect. Thus, all mice that received PT concomitantly with the encephalitogenic challenge developed severe EAE, whilst all mice that received PBS or B-oligomer remained healthy.

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CLAIMS

1. A pharmaceutical composition for the treatment of autoimmune diseases comprising as active ingredient the B-oligomer of pertussis toxin (PT), an individual subunit S2, S3, S4 or S5 of said B-oligomer, or a combination of said subunits, and a pharmaceutically acceptable carrier.

2. A pharmaceutical composition according to claim 1 for the treatment of an autoimmune disease selected form the group comprising multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus and graft-versus-host disease.

3. A pharmaceutical composition according to claim 1 or 2 wherein the active ingredient is the B-oligomer of pertussis toxin.

4. A pharmaceutical composition according to claim 1 or 2 wherein the active ingredient is the subunit S2 or S3 or a mixture of S2+S3 or of S2+S3+S5.

5. A pharmaceutical composition according to claim 1 or 2 wherein the active ingredient is a heterodimer S2/S4 or S3/S4 or a mixture of both.

6. Use of a protein selected from the group comprising the B-oligomer of pertussis toxin, an individual subunit S2, S3, S4 and S5 of said B-oligomer, or a combination of said subunits, for the preparation of a medicament for the treatment of autoimmune diseases.

7. Use according to claim 6 wherein the autoimmune disease is selected from the group comprising multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus and graft-versus-host disease.

8. Use according to claim 7 wherein the autoimmune disease is multiple sclerosis.

9. Use according to any one of claims 6 to 8 wherein the protein is the B-oligomer of pertussis toxin.

10. Use according to any one of claims 6 to 8 wherein the protein is the subunit S2 or S3 of the B-oligomer of pertussis toxin or a mixture S2+S3 or S2+S3+S5.

11. Use according to any one of claims 6 to 8 wherein the protein is a heterodimer S2/S4 or S3/S4 or a mixture of both.

12. A method for the treatment of a patient afflicted with an autoimmune disease which comprises administering to said patient an effective amount of a protein selected from the group comprising the B-oligomer of pertussis toxin, an individual subunit S2, S3, S4 or S5 thereof, or a combination of said subunits.

13. A method according to claim 12 wherein the autoimmune disease is selected from the group comprising multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus and graft-versus-host disease.

14. A method according to claim 13 for the treatment of a patient afflicted with multiple sclerosis which comprises administering to said patient an effective amount of a protein selected from the B-oligomer of pertussis toxin, the subunit S2 or S3 thereof, or a mixture of the subunits S2+S3 or S2+S3+S5, or a heterodimer S2/S4 or S3/S4 or a mixture of both.

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1   TCC ACG CCA GGC ATC GTC ATT CCG CCG CAG GAA CAG ATT ACC CAG     45
    Ser Thr Pro Gly Ile Val Ile Pro Pro Gln Glu Gln Ile Thr Gln
                    5                  10                  15

46  CAT GGC AGC CCC TAT GGA CGC TGC GCG AAC AAG ACC CGT GCC CTG     90
    His Gly Ser Pro Tyr Gly Arg Cys Ala Asn Lys Thr Arg Ala Leu
                20                  25                  30

91  ACC GTG GCG GAA TTG CGC GGC AGC GGC GAT CTG CAG GAG TAC CTG    135
    Thr Val Ala Glu Leu Arg Gly Ser Gly Asp Leu Gln Glu Tyr Leu
            35                  40                  45

136 CGT CAT GTG ACG CGC GGC TGG TCA ATA TTT GCG CTC TAC GAT GGC    180
    Arg His Val Thr Arg Gly Trp Ser Ile Phe Ala Leu Tyr Asp Gly
        50                  55                  60

181 ACC TAT CTC GGC GGC GAA TAT GGC GGC GTG ATC AAG GAC GGA ACA    225
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    65                  70                  75

226 CCC GGC GGC GCA TTC GAC CTG AAA ACG ACG TTC TGC ATC ATG ACC    270
    Pro Gly Gly Ala Phe Asp Leu Lys Thr Thr Phe Cys Ile Met Thr
                80                  85                  90

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    Thr Arg Asn Thr Gly Gln Pro Ala Thr Asp His Tyr Tyr Ser Asn
            95                  100                 105

316 GTC ACC GCC ACT CGC CTG CTC TCC AGC ACC AAC AGC AGG CTA TGC    360
    Val Thr Ala Thr Arg Leu Leu Ser Ser Thr Asn Ser Arg Leu Cys
        110                 115                 120

361 GCG GTC TTC GTC AGA AGC GGG CAA CCG GTC ATT GGC GCC TGC ACC    405
    Ala Val Phe Val Arg Ser Gly Gln Pro Val Ile Gly Ala Cys Thr
    125                 130                 135

406 AGC CCG TAT GAC GGC AAG TAC TGG AGC ATG TAC AGC CGG CTG CGG    450
    Ser Pro Tyr Asp Gly Lys Tyr Trp Ser Met Tyr Ser Arg Leu Arg
    140                 145                 150

451 AAA ATG CTT TAC CTG ATC TAC GTG GCC GGC ATC TCC GTA CGC GTC    495
    Lys Met Leu Tyr Leu Ile Tyr Val Ala Gly Ile Ser Val Arg Val
    155                 160                 165

496 CAT GTC AGC AAG GAA GAA CAG TAT TAC GAC TAT GAG GAC GCA ACG    540
    His Val Ser Lys Glu Glu Gln Tyr Tyr Asp Tyr Glu Asp Ala Thr
    170                 175                 180

541 TTC GAG ACT TAC GCC CTT ACC GGC ATC TCC ATC TGC AAT CCT GGA    585
    Phe Glu Thr Tyr Ala Leu Thr Gly Ile Ser Ile Cys Asn Pro Gly
    185                 190                 195

586 TCA TCC TTA TGC                                             597
    Ser Ser Leu Cys
    199
```

Figure 1

```
1   GTT GCG CCA GGC ATC GTC ATC CCG CCG AAG GCA CTG TTC ACC CAA   45
    Val Ala Pro Gly Ile Val Ile Pro Pro Lys Ala Leu Phe Thr Gln
                    5                  10                  15

46  CAG GGC GGC GCC TAT GGA CGC TGC CCG AAC GGA ACC CGC GCC TTG   90
    Gln Gly Gly Ala Tyr Gly Arg Cys Pro Asn Gly Thr Arg Ala Leu
                20                  25                  30

91  ACC GTG GCC GAA CTG CGC GGC AAC GCC GAA TTG CAG ACG TAT TTG   135
    Thr Val Ala Glu Leu Arg Gly Asn Ala Glu Leu Gln Thr Tyr Leu
                35                  40                  45

136 CGC CAG ATA ACG CCC GGC TGG TCC ATA TAC GGT CTC TAT GAC GGT   180
    Arg Gln Ile Thr Pro Gly Trp Ser Ile Tyr Gly Leu Tyr Asp Gly
                50                  55                  60

181 ACG TAC CTG GGC CAG GCG TAC GGC GGC ATC ATC AAG GAC GCG CCG   225
    Thr Tyr Leu Gly Gln Ala Tyr Gly Gly Ile Ile Lys Asp Ala Pro
                65                  70                  75

226 CCA GGC GCG GGG TTC ATT TAT CGC GAA ACT TTC TGC ATC ACG ACC   270
    Pro Gly Ala Gly Phe Ile Tyr Arg Glu Thr Phe Cys Ile Thr Thr
                80                  85                  90

271 ATA TAC AAG ACC GGG CAA CCG GCT GCG GAT CAC TAC TAC AGC AAG   315
    Ile Tyr Lys Thr Gly Gln Pro Ala Ala Asp His Tyr Tyr Ser Lys
                95                  100                 105

316 GTC ACG GCC ACG CGC CTG CTC GCC AGC ACC AAC AGC AGG CTG TGC   360
    Val Thr Ala Thr Arg Leu Leu Ala Ser Thr Asn Ser Arg Leu Cys
                110                 115                 120

361 GCG GTA TTC GTC AGG GAC GGG CAA TCG GTC ATC GGA GCC TGC GCC   405
    Ala Val Phe Val Arg Asp Gly Gln Ser Val Ile Gly Ala Cys Ala
                125                 130                 135

406 AGC CCG TAT GAA GGC AGG TAC AGA GAC ATG TAC GAC GCG CTG CGG   450
    Ser Pro Tyr Glu Gly Arg Tyr Arg Asp Met Tyr Asp Ala Leu Arg
                140                 145                 150

451 CGC CTG CTG TAC ATG ATC TAT ATG TCC GGC CTT GCC GTA CGC GTC   495
    Arg Leu Leu Tyr Met Ile Tyr Met Ser Gly Leu Ala Val Arg Val
                155                 160                 165

496 CAC GTC AGC AAG GAA GAG CAG TAT TAC GAC TAC GAG GAC GCC ACA   540
    His Val Ser Lys Glu Glu Gln Tyr Tyr Asp Tyr Glu Asp Ala Thr
                170                 175                 180

541 TTC CAG ACC TAT GCC CTC ACC GGC ATT TCC CTC TGC AAC CCG GCA   585
    Phe Gln Thr Tyr Ala Leu Thr Gly Ile Ser Leu Cys Asn Pro Ala
                185                 190                 195

586 GCG TCG ATA TGC   597
    Ala Ser Ile Cys
                199
```

Figure 2

```
1   GAC GTT CCT TAT GTG CTG GTG AAG ACC AAT ATG GTG GTC ACC AGC    45
    Asp Val Pro Tyr Val Leu Val Lys Thr Asn Met Val Val Thr Ser
                     5                  10                  15

46  GTA GCC ATG AAG CCG TAT GAA GTC ACC CCG ACG CGC ATG CTG GTC    90
    Val Ala Met Lys Pro Tyr Glu Val Thr Pro Thr Arg Met Leu Val
                20                  25                  30

91  TGC GGC ATC GCC GCC AAA CTG GGC GCC GCG GCC AGC AGC CCG GAC   135
    Cys Gly Ile Ala Ala Lys Leu Gly Ala Ala Ala Ser Ser Pro Asp
            35                  40                  45

136 GCG CAC GTG CCG TTC TGC TTC GGC AAG GAT CTC AAG CGT CCC GGC   180
    Ala His Val Pro Phe Cys Phe Gly Lys Asp Leu Lys Arg Pro Gly
            50                  55                  60

181 AGC AGT CCC ATG GAA GTC ATG TTG CGC GCC GTC TTC ATG CAA CAA   225
    Ser Ser Pro Met Glu Val Met Leu Arg Ala Val Phe Met Gln Gln
            65                  70                  75

226 CGG CCG CTG CGC ATG TTT CTG GGT CCC AAG CAA CTC ACT TTC GAA   270
    Arg Pro Leu Arg Met Phe Leu Gly Pro Lys Gln Leu Thr Phe Glu
            80                  85                  90

271 GGC AAG CCC GCG CTC GAA CTG ATC CGG ATG GTC GAA TGC AGC GGC   315
    Gly Lys Pro Ala Leu Glu Leu Ile Arg Met Val Glu Cys Ser Gly
            95                  100                 105

316 AAG CAG GAT TGC CCC                                         330
    Lys Gln Asp Cys Pro
            110
```

Figure 3

```
1   GCC GGC TTG CCG ACC CAT CTG TAC AAG AAC TTC ACT GTC CAG GAG   45
    Ala Gly Leu Pro Thr His Leu Tyr Lys Asn Phe Thr Val Gln Glu
                    5                  10                  15

46  CTG GCC TTG AAA CTG AAG GGC AAG AAT CAG GAG TTC TGC CTG ACC   90
    Leu Ala Leu Lys Leu Lys Gly Lys Asn Gln Glu Phe Cys Leu Thr
                20                  25                  30

91  GCC TTC ATG TCG GGC AGA AGC CTG GTC CGG GCG TGC CTG TCC GAC   135
    Ala Phe Met Ser Gly Arg Ser Leu Val Arg Ala Cys Leu Ser Asp
                35                  40                  45

136 GCG GGA CAC GAG CAC GAC ACG TGG TTC GAC ACC ATG CTT GGC TTT   180
    Ala Gly His Glu His Asp Thr Trp Phe Asp Thr Met Leu Gly Phe
                50                  55                  60

181 GCC ATA TCC GCG TAT GCG CTC AAG AGC CGG ATC GCG CTG ACG GTG   225
    Ala Ile Ser Ala Tyr Ala Leu Lys Ser Arg Ile Ala Leu Thr Val
                65                  70                  75

226 GAA GAC TCG CCG TAT CCG GGC ACT CCC GGC GAT CTG CTC GAA CTG   270
    Glu Asp Ser Pro Tyr Pro Gly Thr Pro Gly Asp Leu Leu Glu Leu
                80                  85                  90

271 CAG ATC TGC CCG CTC AAC GGA TAT TGC GAA   300
    Gln Ile Cys Pro Leu Asn Gly Tyr Cys Glu
                95                  100
```

Figur 4

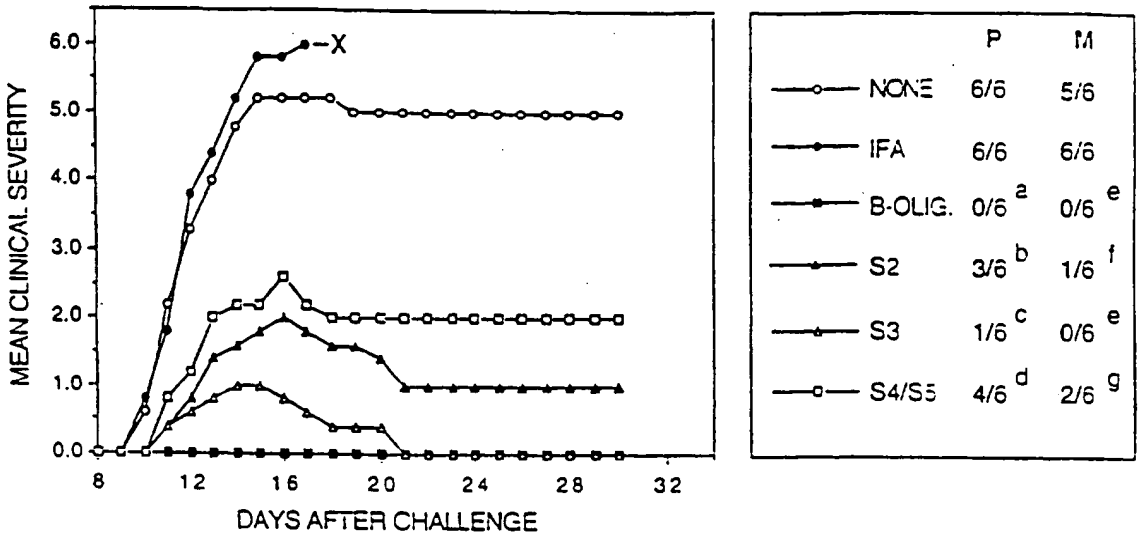


Figure 5

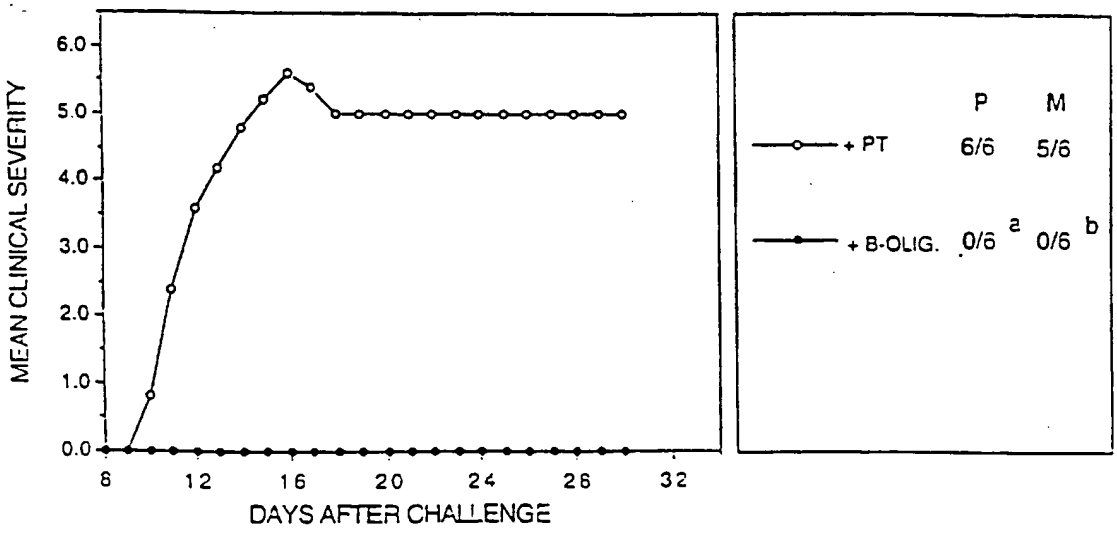
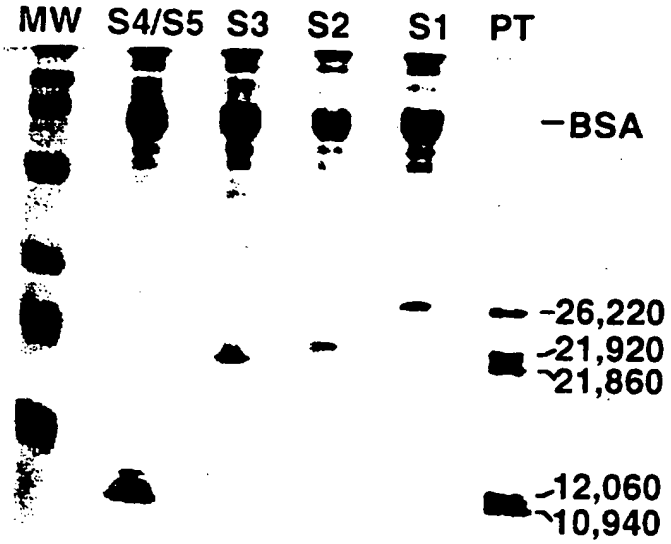


Figure 6

FIG. 7

GEL-PURIFIED PT SUBUNITS
(SILVER-STAINED SDS-GEL)



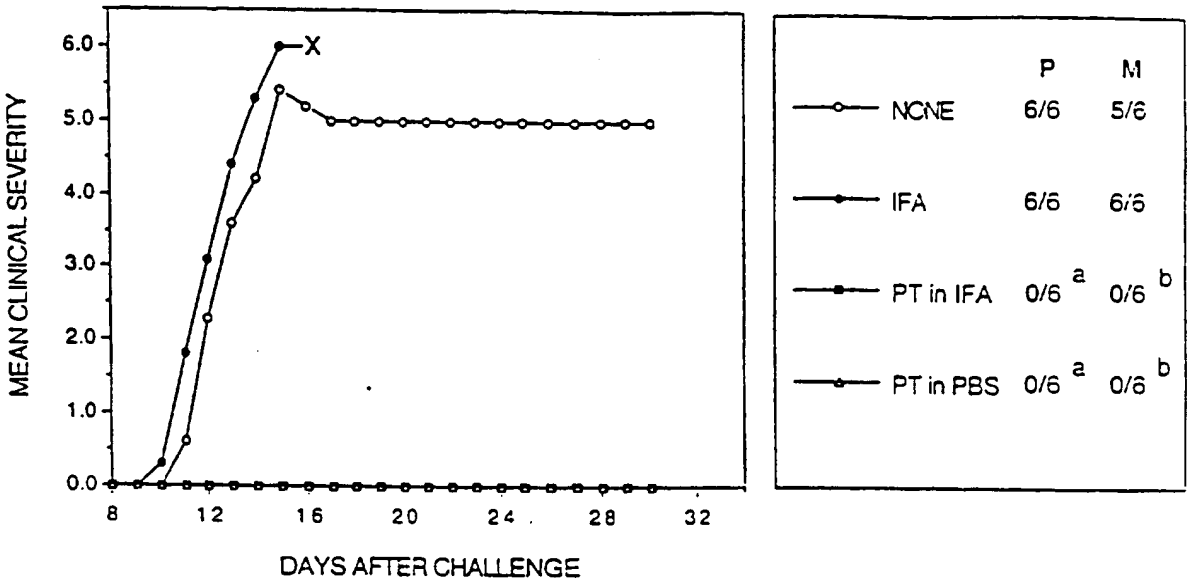


Figure 8

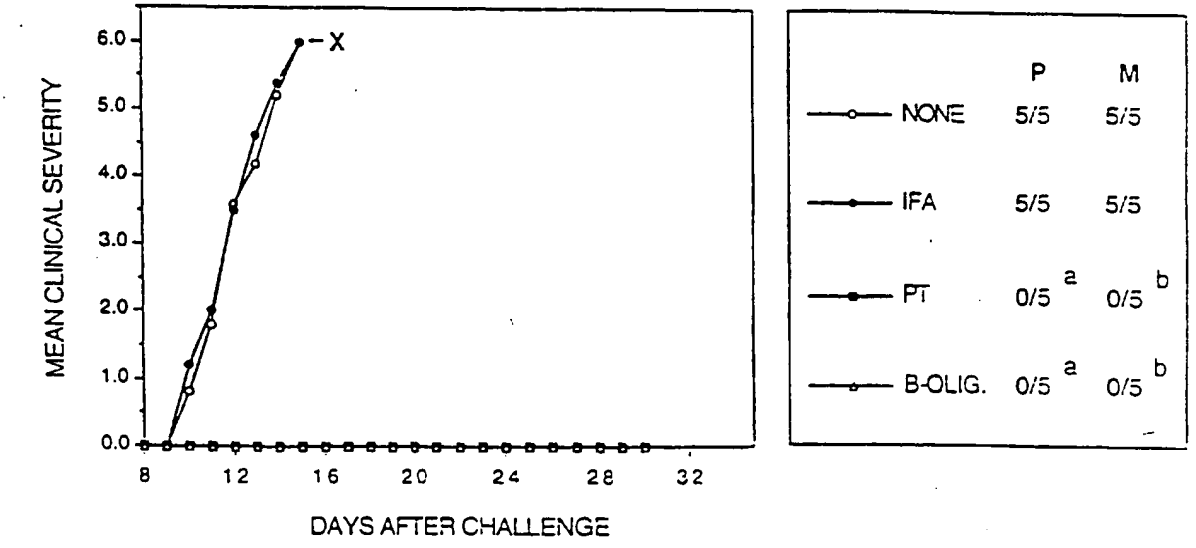


Figure 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/16450

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/16, 39/10; C07K 14/235
US CL :424/240.1; 514/2; 530/350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/190.1, 240.1; 514/2, 12; 530/350, 825

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Infection And Immunity, Volume 59, Number 12, issued December 1991, Nencioni et al, "Properties of the B Oligomer of Pertussis Toxin", pages 4732-4734, see page 4732, column 2, last two paragraphs.	1-4, 6-10
X	JP, A, 58-59925 (KAKEN YAKUKAKOU KK) 09 April 1983, see the abstracts.	1-4, 6-10, 12, 13
A	The Journal Of Biological Chemistry, Volume 258, Number 11, issued 10 June 1983, Tamura et al, "A Role of the B-Oligomer Moiety of Islet-activating Protein, Pertussis Toxin, in Development of the Biological Effects on Intact Cells", pages 6756-6761, see page 6759, paragraph bridging columns 1 and 2, and page 6760, column 2, first full paragraph.	1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 14 MARCH 1996	Date of mailing of the international search report 25 MAR 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JEFFREY E. RUSSEL Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/16450

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	European Journal Of Immunology, Volume 23, issued 1993, Ben-Nun et al, "Protection against autoimmune disease by bacterial agents. II. PPD and pertussis toxin as proteins active in protecting mice against experimental autoimmune encephalomyelitis", pages 689-696.	1-14
X	WO, A, 92/19646 (THE ROCKEFELLER UNIVERSITY) 12 November 1992, see page 4, line 18 - page 5, line 14, page 23, line 20 - page 24, line 7, page 32, lines 1-3.	1-4, 6-10, 12-14
X	US, A, 4,845,036 (BURNS ET AL) 04 July 1989, see column 1, line 62 - column 2, line 16, column 2, lines 46-47, column 3, lines 1-3.	1-4, 6-10
A,P	US, A, 5,453,272 (HEERZE ET AL) 26 September 1995, see column 14, lines 20-26, column 18, lines 43-45.	1-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/16450

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, DIALOG
search terms: bordetella pertussis, b oligomer, subunit, s2, s3, s4, s5, autoimmune, multiple sclerosis, arthritis, lupus, diabetes, graft-versus-host disease